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Evaluation of Phosphate Solubilising Potential of Some Endophytic Fungi under Solid and Liquid State

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Abstract

Twenty six fungi belonging to *Alternaria, Aspergillus, fusarium, Paecilomyces, Penicillium sp*. and few sterile mycelia were evaluated for the phosphate solubilising properties under solid and submerged culture conditions. Most of the *Aspergillus sp.* exhibited good solubilisation activity than other species listed. Plate screening test confirm the highest phosphate solubilisation efficiency by *Sterile mycelium sp. 3* (89.4%) followed by *Aspergillus sp.* 1 (85.7%) and *Fusarium sp.* 1 and 3 (81.25%). Solubilization index calculated for these fungi was ranged between 1.80-1.89 . All fungi were evaluated for phosphate solubilisation efficiency under liquid culture condition supplemented with TCP. *Aspergillus sp.4, Aspergillus sp.8* and *Penicillium sp.1* solubilised 29.6%, 26.7% and 24.5% respectively. Decline in pH of the culture filtrate of 10 day old culture was observed in liquid culture of *Aspergillus sp. 2* (4.59), *Aspergillus sp 4* (4.79) and *Aspergillus sp 8* (4.9). However, no correlation was observed between acid production and phosphate solubilisation. These fungal strains performed differentially under both culture conditions. Hence testing of phosphate solubilisation efficiency of these organisms under both the solid and liquid conditions is suggested in the present study.

Keywords- Fungi,P solubilization,Endophytic, *Aspergillus,Fusarium, Penicillium.*

Introduction

Microbes have capability of solubilizing the inorganic phosphates. (Omar, 1998; Seshadri *et al.,* 2004; Wakelin *et al.,* 2004). Phosphate solubilizing microbes can solubilize and mineralize phosphorus and make it available to plants.The mechanism of phosphorous solubilisation by microbes involves: lowering of pH by biotic production of proton/bicarbonate release, gaseous exchange, chelation of cations and by competing with phosphorous for the adsorption sites in the soil (Nahas, 1996). Some of the inorganic acids (e.g. HCl) are also solubilizing phosphorous (Kim *et al.*, 1997).There are other various mechanisms by

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which microorganisms solubilize inorganic phosphate other than secretion of organic acids (Goldstein, 1995), like production of siderophores (Vassilev *et al.,* 2006) and secretion of some phenolic compounds and humic substances (Patel *et al.,* 2008). However, production of organic acids is considered to be the means by which phosphate compounds are mobilized and able to dissolve the mineral phosphate thereby making it available for the plants (Bhattacharya and Jain, 2000).

Endophytic microorganisms are those microbes present within the intercellular spaces of plant parts without causing any harm and hence are less detrimental to their hosts. However they can enhance plant growth via phosphate solubilization, production of siderophores, by enhancing hyphal growth and mycorrhizal colonization etc. (Barooah *et al.,* 2012).

Several authors have identified the ability of fungi, mainly of *Aspergillus* and *Penicillium* genus, to solubilize phosphates under in vitro conditions (Omar, 1998; Seshadri*et al.,* 2004; Wakelin *et al.,* 2004).Among these organisms are species of *Aspergillus, Penicillium, Talaromyces*, and *Eupenicillium*, which are also considered "key organisms" in the P cycle occurring in the environment (Whitelaw, 2000). Most of them organisms can solubilize inorganic calcium phosphates but have a lower capacity of solubilizing aluminum or iron phosphates (Illmer and Schinner, 1995). Hence; there is keen interest in isolating phosphate solubilizing microbes from novel sources, endophytic sources are significantly important because of their beneficial role in plant growth and development.

Materials and Methods

Source of fungal strains

The endophytic fungal isolates were obtained from Microbial culture collection, Regional Plant Resource Center, Bhubaneswar, Odisha.

Screening of the isolates for Phosphate solubilization

Fungal spores were inoculated to Pikovskaya's agar medium (pH-7.2) and inoculated at 28^0C for 5-7days. Clear zones around the colonies indicated the capacity of phosphate solubilization. On the bases of diameter of clearing halo zones, Solubilization efficiency (SE) was calculated. (Gaur, 1990) and Solubilization index (S.I.) was measured using the formula (Edi-premono et al., 1996).

Quantitative estimation of tricalcium phosphate solubilisation (Vanadophosphomolybdate method, Jackson 1958)

The fungi was inoculated into Pikovskaya's broth medium in triplicates and incubated at 28^0C for 10 days.Mycelium was separated from culture broth after 10 days of incubation. Initial pH and change in pH was noted for all the samples with the help of digital pH meter followed by analysis of P in broth.10 ml of sample is centrifuged at 1500rpm/15 min.5ml sample is mixed with 10 ml reagent and made up to 50 ml with distilled water. The above mixture is incubated for 30 minutes at room temperature. Then O.D. is taken at 420 nm, concentration of phosphate is calculated from standard graph.The amount of soluble phosphate was calculated from standard curve of KH_2PO_4.Absorbance of the developing yellow colour was measured at 420 nm wave length with UV- VIS spectrophotometer (Specord 50).

Results and Discussion

160 fungal strains were tested for phosphate solubilising potential. Among them 26 fungal strains belonging to genera *Alternaria(1), Aspergillus(10), Curvularia(1), Fusarium(3), Paecilomyces(2), Penicillium(2), Sterile mycelium*(7) were found to be having phosphate solubilisation under solid culture. The solubilization efficiency and solubilization index of each isolate based on colony diameter and halozone is presented in Table 1. Phosphate solubilizing organisms formed clear halozones on medium plates which revealed that they can solubilize tricalcium phosphate, the extent of solubilization varies in different isolates which is measurable. The solubilization index ranged from 1.09-1.89 and correlated with the studies of

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Mahamuni *et al.,* 2012 and Alam *et al.,* 2002. Results showed that *Sterile mycelium* sp.3 showed maximum solubilization efficiency and solubilization index (S.E. = 89.4% and S.I. = 1.89) followed by *Aspergillus sp.1* which showed solubilization efficiency and solubilization index (S.E. = 85.7% and S.I. = 1.86). However, *Penicillium sp.* 2 showed the lowest solubilisation efficiency and solubilization index (S.E. = 13 % and S.I. = 1.13).

Table 1: Evaluation of phosphate solubilization activity of different fungi under plate culture conditions

Sl.no.	Organism	SOLUBILIZATION EFFICIENCY (%)	SOLUBILIZATION INDEX (S.I.)
1	*Alternaria sp 1*	40	1.4
2	*Aspergillus sp .1*	85.7	1.86
3	*Aspergillus sp. 2*	67.85	1.68
4	*Aspergillus sp .3*	59.1	1.59
5	*Aspergillus sp.4*	53.57	1.58
6	*Aspergillus sp .5*	37.5	1.375
7	*Aspergillus sp .6*	53.5	1.535
8	*Aspergillus sp. 7*	53.5	1.535
9	*Aspergillus sp. 8*	61.5	1.615
10	*Aspergillus sp. 9*	42.9	1.43
11	*Aspergillus sp. 10*	63.6	1.64
12	*Curvularia sp. 1*	56.6	1.57
13	*Fusarium sp. 1*	81.25	1.81
14	*Fusarium sp. 2*	28	1.28
15	*Fusarium sp. 3*	81.25	1.81
16	*Paecilomyces sp. 1*	60	1.6
17	*Paecilomyces sp. 2*	25	1.25
18	*Penicillium sp. 1*	45	1.45
19	*Penicillium sp. 2*	13	1.13
20	*Sterile mycelium sp. 1*	72.2	1.72
21	*Sterile mycelium sp. 2*	80	1.8
22	*Sterile mycelium sp. 3*	89.4	1.89
23	*Sterile mycelium sp. 4*	36.5	1.365
24	*Sterile mycelium sp. 5*	78.6	1.79
25	*Sterile mycelium sp. 6*	41.6	1.42
26	*Sterile mycelium sp. 7*	28	1.28

ISSN 2349-4603

TABLE 2: Evaluation of phosphate solubilization activity of different fungi under liquid culture conditions

Sl.no.	Organism	pH Reading	% P Solubilized (mean±s.d)
1	*Alternariasp 1*	8.34±0.067	5.4±0.35
2	*Aspergillussp 1*	6.86±0.067	16.5±1.59
3	*Aspergillussp 2*	4.59±0.025	16.6±1
4	*Aspergillussp 3*	5.93±0.11	12.2±0.06
5	*Aspergillussp 4*	4.79±0.031	29.6±2.06
6	*Aspergillussp 5*	6.72±0.075	21.6±0.4
7	*Aspergillussp 6*	5.65±0.05	20.7±0.25
8	*Aspergillussp 7*	5.76±0.06	11.8±0.42
9	*Aspergillussp 8*	4.9±0.025	26.7± 0
10	*Aspergillussp 9*	7.03±0.076	12.2±0.35
11	*Aspergillussp 10*	5.86±0.04	13±0.17
12	*Curvulariasp 1*	7.35±0.025	5±0.06
13	*Fusariumsp 1*	7.84±0.03	3.3±0.23
14	*Fusariumsp 2*	8.33±0.071	2.7±0.1
15	*Fusariumsp 3*	8.53±0.11	4.5±0.95
16	*Paecilomycessp 1*	8.27±0.31	4.8±0.35
17	*Paecilomycessp 2*	8.67±0.166	1±0.17
18	*Penicilliumsp 1*	7.59±0.155	24.5±5.13
19	*Penicilliumsp 2*	8.74±0.032	1.5±0.91
20	*Sterile mycelium sp 1*	6.7±0.1	8.3±2.08
21	*Sterile mycelium sp 2*	8.17±0.127	9.7±2.55
22	*Sterile mycelium sp 3*	6.45±0.12	2.8±0.1
23	*Sterile mycelium sp 4*	6.31±0.23	15.43±1.75
24	*Sterile mycelium sp 5*	6.35±0.03	8.3±0.1
25	*Sterile mycelium sp 6*	7.13±0.042	14.3±1.44
26	*Sterile mycelium sp 7*	6.43±0.03	9.4±0

A significant pH change was observed as compared to uninoculated control incubated for a period of 10 days as recorded in Table 2. The pH ranged from 4.59-8.74. Among all the isolates, *Aspergillus sp.*2 showed drop in pH to 4.59 from 7.1 (control) followed by *Aspergillus sp.4* which showed pH 4.79.

The phenomenon of P solubilization is indicated from the lowering of pH of the medium. Solubilized P obtained from different isolates in percentage demonstrated the signatory importance of the efficient phosphate solubilizing strains. Percent P solubilised in presence of TCP in liquid culture ranged from 1% -29.6% which clearly demarcates

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the efficiency of P solubilisation between the isolates taken up for our study. *Aspergillus sp.*4 showed a maximum of 29.6% solubilization in liquid culture conditions followed by *Aspergillus sp.* 8 of about 26.7% solubilization. However, *Paecilomyces sp. 2* showed 1% solubilisation in liquid culture conditions. Most of the *Aspergillus sp.* showed good p solubilization activity whereas *Fusarium sp., Paecilomyces sp.* and *Curvularia sp*. had poor P solubilisation in broth culture conditions.

The ability to solubilize phosphorus varies in most of the strains when compared with medium conditions. It is evident from our findings that the fungi exhibited good solubilization potential in solid medium had shown poor solubility under liquid culture as seen in case of *Fusarium sp.1* (S.E. = 81.25% and S.I. = 1.81) with 3.3% solubilization and *Sterile mycelium sp.3* (S.E. = 89.4% and S.I. = 1.89) with 1% solubilization in liquid broth. Similiarly, *Aspergillus sp.1* having (S.E. = 85.7% and S.I. = 1.86) showed 16.5 % solubilization under plate and liquid culture conditions. Therefore, production of halo zone on solid medium showing larger halo zones may not solubilise more phosphorus in liquid medium. Hence isolation of PSF from visible zone surrounding the fungal colony on agar plate only cannot be considered as proper parameter of distinguishing efficient strains. However, in vivo studies can only justify the potential of any phosphate solubilizing strain in promotion of plant growth and their establishment. Since, these fungal strains performed differentially under both culture conditions. Hence testing of phosphate solubilisation efficiency of these organisms under both the solid and liquid conditions is suggested in the present study.

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